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PERFORMANCE REPORT

FEBRUARY 1995- FEBRUARY 1997

US OSTEO- STS-76

NASA grant NAG 2-981

Grant Title:
"Effects of microgravity on bone cell gene expression"

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ABSTRACT

Osteoporosis is a generic term used to describe various bone diseases that result in fractures of the vertebrae, wrist hip, humerus and tibia. Osteoporosis is common in older adults, in patients with excess glucocorticoid as in Cushings syndrome or in people treated for asthma with steroids and in healthy astronauts that are exposed to microgravity for extended duration. Our studies concentrate on the basic mechanisms that regulate new bone growth and the relationship of growth to gravity environment. Using Biorack hardware we activated osteoblast cells to grow in the same microgravity experienced by the crew as well as in an onboard 1g control in order to investigate alterations in bone growth and molecular patterns of gene expression. Determining how gravity affects growth and gene expression will help us determine environmental or pharmaceutical changes that would allow normal bone growth during long term space exploration. We have just published our findings from our experiment on STS-56 showing that microgravity interferes with normal osteoblast growth activation and causes reduced PGE_2 synthesis after 5 days.

Effect of Shuttle Launch Profile on Gene Expression - Baseline data collection for OSTEO The lack of gravity in spaceflight is a key factor in bone loss since the necessary mechanical strain induced by gravity is missing in 0g. These experiments have shown that mechanical strain caused by the hypergravitational pull of launch results in the elevation of c-fos in serum deprived osteoblasts. c-fos is one of the earliest expressed genes during activation of bone growth. Serum-deprived mouse osteoblastic cells (MC3T3-E1) were centrifuged in the AMES centrifuge in a gravity profile simulating a Space Shuttle launch (maximum of 3g). mRNA levels for 9 genes involved in bone growth and maintenance were determined using RT-PCR. 30 minutes after centrifugation the mRNA for early response gene, *c-fos*, was significantly increased 89% ($P<0.05$). The *c-fos* induction was transient and returned to control levels after 3 hours. mRNA for the mineralization marker gene, osteocalcin, was significantly decreased to 44% of control levels ($P<0.005$), 3 hours after centrifugation. No changes in mRNA levels were detected for *c-myc*, TGFb1, TGFb2, cyclophilin A, or actin. In addition, no change in the steady state synthesis of prostaglandin E_2 (PGE_2) was detected, probably due to lack of lipid substrates in serum deprived cells. This suggests that at least a portion of the increase in *c-fos* mRNA in response to gravitational loading is a direct result of mechanical stimulation.

These results indicate that small magnitude mechanical loading, such as that experienced during a Shuttle launch, can alter mRNA levels in quiescent osteoblastic cells. This is probably one of the mechanisms by which exercise augments bone growth

Analysis of the vibrational data launch profile is not yet complete. However, the preliminary results are showing significant differences in the magnitude of gene expression and in the number of genes activated during vibration when compared to gravity launch profile. These data strongly suggest that NASA should make every effort to dampen the vibrational component of launch on biological samples. In addition, with changes in gene expression occurring as a result of launch, an on onboard 1g control becomes critical to proper analysis of the samples.

STS-76 FLIGHT SAMPLES

RNA RESULTS:

The OSTEO experiment aims to analyze how microgravity effects bone loss by investigating alterations in select gene expression patterns in the prostaglandin pathway. We are analyzing key genes responsible for osteoblast growth and homeostasis. Expression patterns are being analyzed in osteoblasts exposed to microgravity using rtPCR technology. In these studies we are be able to determine if the microgravity environment can allow normal bone growth and to determine if changes in prostaglandins or their pathway enzymes play a role in the bone loss seen in spaceflight. The analysis of the samples is currently in progress. We have collected intact mRNA from the flight (0 and 1g) and ground (GR) samples and we have

triplicate mRNA samples from our 3, 29 and 54 hour timepoints for all 0g flight samples. The amount of total RNA collected ranged from 2.4 to 15.3 ug per sample.

We consider our RNA recovery to be 100% successful since we have a minimum of three mRNA samples for each timepoint and were able to get statistically significant data from 0g flight samples. We have preliminary data on our 9 target genes. Preliminary analysis of the RNA expression shows significant differences between flight and ground.

Final analysis is still in progress since funding meant for this phase was diverted to cover costs for the STS-81 flight since NASA withheld moneys due early 1996 for the STS-81 flight until late 1996. There is still a lot of ground base work to complete before we can make definitative statements on effects of microgravity and before we publish. We have asked for a no cost extension for this grant to allow us to finish the studies.

MORPHOLOGY STUDIES:

The OSTEO experiment recovery of the formaldehyde fixed cells is also 100% successful. The off nominal operations during flight did not in any way harm our results or cause loss of science data. In one case, the cells were fixed at t=0 instead of 54 hours, but we were able to use this time point for our 0 time morphology control. Luckily, the lost sample was paired with a plunger box with 4 samples, so our statistical data base was not damaged by the off nominal operations. We are seeing statistical differences in morphology of the cells grown in microgravity. In the methods section, we show a color plate of these cells, showing differences in morphology, cell size and nuclear shape and size in the 1g and 0g grown osteoblasts. These data are being prepared in manuscript form for submission for publication.

CHEMICAL ANALYSIS OF MEDIA:

The analysis of the media from the samples is not yet complete, however preliminary data confirm our previous results on STS-56. With the flight samples using less glucose when compared to ground samples. Analysis is continuing on this and other media components. Analysis for prostaglandins are finished and we find that the PGEs are increased in flight samples.

OVERALL ASSESSMENT OF RETRIEVED SAMPLE AND DATA QUALITY:

Excellent results with no conditions compromising samples. For the first time substantial quantities of RNA have been collected from cells activated and collected during spaceflight to make statistical observations on gene expression during osteoblast growth activation in microgravity. We are extending our studies to include mRNA analysis of mineralization (differentiation) of cells in flight. Morphological studies confirm and extent our prior observations on changes of morphology changes in spaceflight.

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3. Hughes-Fulford, M. Review of the Biological Effects of Weightlessness on the Human Endocrine System, Receptor, 3(3):145-154, 1993.

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14.Lewis, ML and Hughes-Fulford, M. Cellular Responses to Microgravity, Chapter 3, Textbook for the International Space University, ed. Suzanne Churchill. Harvard University, In Press 1997

< CONFIRMATION REPORT >

02-18-1997(TUE) 15:51

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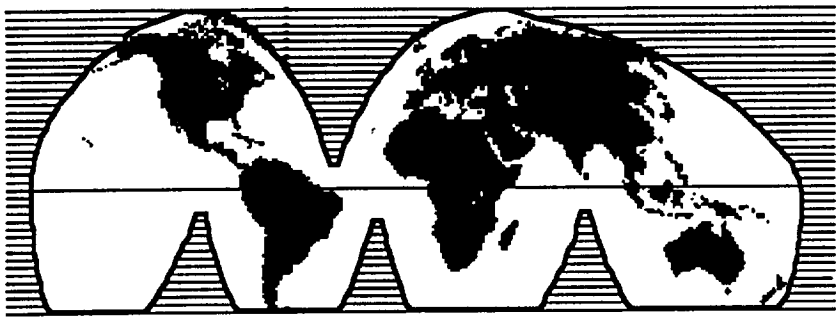

FAX IN BRIEF

TO: Barrie Caldwell, NASA

FAX: 415 6044646
FROM: Hughes-Fulford

PAGES (INCLUDING COVER): 6

Friday, October 18, 1996

I have noticed that no one has received the entire copy, therefore I have abbreviated the report to 5 pages and am re-faxing. Please notify me if you do not receive all 5 pages plus cover sheet.

Millie